IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION TRANSMITTAL LETTER

ATTORNEY DOCKET HEBVR-5

To: THE COMMISSIONER OF PATENTS AND TRADEMARKS

Washington D.C. 20231

Transmitted herewith for filing is the patent application of

Howard L. Elford

For: THERAPEUTIC PROCESS FOR INHIBITING NF-κB

Sir:- Enclosed are

(1) A copy of the specification and claims.

(2) Declaration and Power of Attorney

(3) Copy of Verified Statement to Establish Small Entity Status, filed with prior Provisional Application.

The filing fee is calculated as follows:

	No. filed	No. Extra	Rate	Fee
Total Claims	8-20	0	$11	$0
Independant Claims	1-3	0	$41	$0
			Basic Fee =	$790
			Total Filing Fee =	$790
			Small Entity Total =	$395

A check in the amount of $395 is enclosed.

Signature: Atty. of Record James L Rowe

James L. Rowe

Attorney for Applicants

Reg. No. 18,448

7775 Spring Mill Road

Indianapolis IN 46260

317/251-0077

Date: July 28, 1998

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

AMENDMENT TRANSMITTAL LETTER

Commissioner of Patents and Trademarks

Washington DC 20231

Sir:

Enclosed is an amendment to the accmopanying application (formerly Provisional Application No. 60/054,230, filed 7/30/1997)

Atty. No. HEBVR-5

With the entry of the amendment,

	No. Filed	No. Extra	Rate	Fee
Toatal Claims	11-20	0	$11	$0
Indep. Claims	1-3	0	$41	$0

James Z Rowe

Attorney for Applicant

317-251-0077

7775 Spring Mill Rd.

Indianapolis IN 46260

Date July 28, 1998

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

AMENDMENT

Commissioner of Patents and Trademarks

Washington DC 20231

Sir:-

Please amend the accompanying application identified as HEBVR-5, formerly Provisional Application No. 60/054,230. as follows,

In the Claims

Add the following claims

9) A process according to Claim 1 in which the external agency activating NF-κB is the result of a tissue transplant, an organ transplant or a cell transplant in a mammal,

10) A process according to Claim 1 in which the external agency activating NF-κB is arteroisclerosis.

!!) A process according to Claim 1 in which the external agency activating NF-κB is diabetes.

Entry of the above amendment is respectfully requested.

Respectfully submitted,

James L. Rowe

Attorney for Applicant

Reg. No. 18,448

Phone: 317-251-0077 7775 Spring Mill Road

Dated. July 28, 1998 Indianapolis IN 46260

HEBVR-5

THERAPEUTIC PROCESS FOR INHIBITING NF-κB

CROSS-REFERENCE

Provisional Application No. 60/054,230, filed 7/30/97

BACKGROUND OF THE APPLICATION

Eukaryotic cells contain a number of families of transcription factors which serve to rapidly induce expression of a variety of genes in response to extracellular stress or physiological signaling pathways. One important family of transcription factors mediating these responses is a factor named nuclear factor kappa B (NF-κB) and is composed of the NF-κB/Rel proteins. This factor, when in an active state, in turn activates genes involved in the mammalian body's response to inflammation, infection and stress. NF-κB is ubiquitously expressed but appears to play an important role in the etiology and progress of inflammatory disease, both chronic and acute, according to Barnes and Karin. NF-κB is rapidly activated by a wide variety of stimuli including cytokines, protein kinase C activators, viruses, ultraviolet radiation, immune stimuli and agents inducing oxidative stress leading to the production of reactive oxygen intermediates. It is believed that all of these act by means of specific protein kinases that degrade IκB whose biological role is to bind NF-κB and keep it in the inactive

state in the cytoplasm. Antioxidants are also known to block the action of protein kinases indicating that reactive oxygen species may play an intermediary role. When IκB is phosphorylated, it is degraded by proteolytic reactions allowing the NF-κB to enter the nucleus and bind to DNA and transactivate target genes. The redox status of the cell appears to control the presence of IκB and therefore the activity of NF-κB. Antioxidants have been shown to inhibit the oxidative stress activation of NF-κB. Among the genes whose expression is increased by NF-κB are nitric oxide synthetase and cyclooxygenase-2, the latter being responsible for increased production of prostaglandins and thromboxane. NF-κB also regulates the expression of several genes that encode adhesion molecules which in turn recruit inflammatory cells.

Additionally, NF-κB plays a crucial role in the intracellular efficiency of gene expression and replication of the human immunodeficiency virus (HIV-1). Thus, inhibition of NF-κB could also play a role in the treatment of HIV-1 and other viral agents.

Finally, NF-κB status appears to play an important role in cancer treatment. For example, the discovery of Tumor Necrosis Factor (TNF) was hailed as a potential giant step in the treatment of cancer. It was found early on, however, that TNF did not kill most types of cancer cells. Apparently, TNF

triggers one intracellular pathway that leads the cell to commit "suicide," in a process called apoptosis, and simultaneously triggers another that activates a key molecule that blocks this pathway. This second pathway involved genes that were turned on by NF-κB which protected the cancer cells from TNF killing. Recently four separate groups have uncovered information that supports this hypothesis. First, Baltimore and co-workers discovered that mice lacking NF-κB die before birth, apparently from massive die-off of liver cells. This finding implied that NF-κB protects embryonic liver cells from committing suicide. Next, Sonenschein's group found that inhibiting NF-κB causes the B-cells of the immune system to die of apoptosis. These results were followed up by papers from the Baltimore group, from Verma's group at the Salk Institute and from Baldwin's group at University of North Carolina. Baltimore's group compared the effect of TNF on cells from treated mice lacking NF-κB and cells from normal mice. The cells from the normal mice survived but the cells from the mice lacking NF-κB died. Both other groups treated a variety of cells (tumor and non-tumor) with a mutant form of IkB that acts to keep NF-κB irreversibly shackled in the cells cytoplasm. Cells treated with IkB were all killed by TNF. It has been found that other oncolytic agents act on tumor cells in the same way as TNF; i.e., radiation and danorubicin.

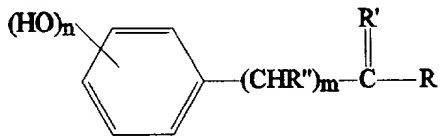
Therefore, the inhibition of NF-κB may enhance the anticancer activity of a number of chemotherapeutic agents that cause cell damage leading to cell suicide via the apoptotic process. Therefore we are providing a superior therapeutic process for NF-κB inhibition.

OBJECTS OF THE INVENTION

It is an object of the invention to provide a therapeutic process from the description which follows.

DESCRIPTION OF THE INVENTION

In fulfillment of the above and other objects, this invention provides a therapeutic process for the inhibition of NF-κB in mammals in whose cells NF-κB has been activated which comprises administering to a mammal in whose cells NF-κB has been activated and in need of treatment, an NF-κB inhibitory amount of a free-radical scavenging drug of the following formula:



wherein n is 2-5, m is 0 or 1, R is NH_2, NHOH, OC_{1-3} alkyl or 0-phenyl, R′ is 0, NH or NOH and R" is H or OH. Also included are the pharmaceutically acceptable salts of compounds according to the above formula where chemically feasible. Also included

within the scope of this invention are the phenolic acetyl derivatives of compounds according to the above formula. Such acetyl derivatives act as "pro-drugs" in that they are converted by the mammalian body to the corresponding compound having entirely unesterified phenolic hydroxyls, which are the therapeutically active drugs.

Illustrative of the polyhydroxy-substituted phenyl ring in the above formula are included 2,3-dihydroxyphenyl, 3,4-dihydroxyphenyl, 2,5-dihydroxyphenyl, 2,6-dihydroxyphenyl, 2,3,4-trihydroxyphenyl, 2,3,5-trihydroxyphenyl, 3,4,5-trihydroxyphenyl, 2,4,5-trihydroxyphenyl, 2,3,4,5-tetrahydroxyphenyl, pentahydroxyphenyl and the like groups.

In the above formula, when m is 1 and R" is H, a phenylacetic acid derivative is denominated. When m is 1 and R" is OH, a mandelic acid derivative is represented. When m is 0, R is NHOH and R′ is O, an N-hydroxybenzamide (formerly, a benzohydroxamic acid) is represented; when R is NH_2 and R′ is NH, a benzimidamide (formerly a benzamidine) is represented; when R is NHOH and R′ is NH, an N-hydroxy benzimidamide (formerly a benzamidoxime) is shown; when R is NHOH and R′ is NOH, an N,N′-dihydroxy benzimidamide (formerly an hydroxyamidoxime) is represented; and when R is O-alkyl or O-phenyl and R′ is NH, the resulting compounds are benzimidates (rather than benzamidates as

previously). In the above formula, when R is OC_{1-3} alkyl, the alkyl groups represented include methyl, ethyl, isopropyl and n-propyl.

Compounds represented by the above formula are fully illustrated in United States Patents 4,252,322, 4,623,659, references cited therein. In particular, the compounds listed in Cols. 2 and 3 of United States Patent 4,623,659 illustrate the scope of the compounds represented by the above formula (always remembering that the approved nomenclature for these structures has changed since 1983 when the application which resulted in that patent was filed) and the disclosure of United States Patent 4,623,659 is incorporated herein, and made a part of, by reference.

It will be apparent to those skilled in the art that other free radical scavengers in addition to those enumerated above would also be operative in the processes of this invention and are therefore included within its scope.

Three extremely active NF-κB inhibitors represented by the above formula are Didox (N,3,4-trihydroxybenzamide); Trimidox (N,3,4,5-tetrahydroxybenzamide) and Amidox (N,3,4-trihydroxybenzimidamide).

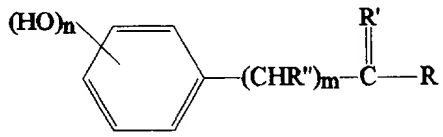
Compounds represented by the above formula may be administered in saline to mammals in whom NF-κB has been

triggered by inflammation, a viral disease, radiation or an anticancer drug, via the intraperitoneal, intravenous, intramuscular, intradermal or intrathecal routes in accordance with the skill of the art. Similarly, it is well within the skill of the art when coupled with published dosages in connection with other diseases in which treatment with a free-radical scavenger might be beneficial to prepare oral medications containing Didox, Amidox, or Trimidox as the above drug, i.e., tablets, filled gelatin capsules, or liquid formulations. As will be apparent also to those skilled in the art, the effective dose levels will vary according to the mode of administration. For example, oral dose levels would be higher, and intravenous or intramuscular levels lower in general than intraperitoneal dose levels. Drug carriers may also be employed and the NF-κB inhibiting agents of this invention can be combined in a combination dosage forms with, or be administered at the same time as, other inhibitory agents. Phenol-acetylated compounds according to the above formula, although called "pro-drugs" herein, can also be considered as a special type of drug carrier.

CLAIMS

I Claim:

1) A process for inhibiting NF-κB in a mammalian cell in which NF-κB has been activated by an agency external to said cell which comprises administering to the mammal in whose cells NF-κB has been activated an NF-κB inhibiting amount of a drug represented by the formula:



wherein n is 2-5, m is 0 or 1, R is NH_2, NHOH, OC_{1-3} alkyl, or 0-phenyl, R′ is 0, NH or NOH, R" is H or OH and pharmaceutically-acceptable acid-addition salts and acylated phenol derivatives thereof.

2) A process according to Claim 1 in which the external agency activating NF-κB is an inflammatory process includes, but is not limited to, a cytokine, an activator of protein kinase B, a virus or an oxidant.

3) A process according to claim 1 in which the external agency activating NF-κB is a drug or radiation administered to the host mammal in a chemotherapeutic process used in the treatment of cancer.

4) A process according to Claim 1 in which the administered NF-κB inhibitor is a free-radical scavenger.

5) A therapeutic process according to Claim 1 in which the NF-κB inhibitor is N,3,4- trihydroxybenzamide.

6) A therapeutic process according to Claim 1 in which the NF-κB inhibitor is N,3,4,5-tetrahydroxybenzamide.

7) A therapeutic process according to Claim 1 in which the NF-κB inhibitor is N,3,4-tetrahydroxybenzimidamide.

8) A therapeutic process according to Claim 1 in which the NF-κB inhibitor is a ribonucleotide reductase inhibitor.

DECLARATION AND POWER OF ATTORNEY

Docket No. HHEBVR-5

As the below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor of the invention entitled

THERAPEUTIC PROCESS FOR INHIBITING NF-κB

which is attached hereto.

I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims.

I acknowledge my duty to disclose information which is material to the examination of this application in accordance with 37 CFR 1.56.

I hereby claim the benefit under 35 USC 119(E) of the following United States Provisional Application:

Application No. 60/054,230 filed 7/30/97

POWER OF ATTORNEY I hereby appoint James L. Rowe to prosecute this application, and transact all business in the United States Patent and Trademark Office connected herewith. All correspondance and telephone calls should be addressed to James L. Rowe, Reg. No. 18,448, 7775 Spring Mill Road, Indianapolis IN 46260; Phone: 317-251-0077

I hereby declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true; andd further that these statements were made with with the knowledge that willful false statements and the like so made are punishable under Section 1001 of Title 18 of the United State Code. .

Full name of inventor: Howard L. Elford
Signature of inventor Howard L. Elford

date: July 28, 1998

Post Office address: 3313 Gloucester Road
Richmond VA 23227

Citizenship: United States

PTO/SB/09 (10-96)
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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
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VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(b))—INDEPENDENT INVENTOR

Docket Number (Optional): HEBRV-5

Applicant or Patentee: Howard L. Elford

Application or Patent No.: Based on provisional application No. 60/054,230

Filed or issued: 7/30/97

Title: THERAPEUTIC PROCESS FOR INHIBITING NF-κB

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in:

- [X] the specification filed herewith with title as listed above.
- [X] the application identified above.
- [ ] the patent identified above.

I have not assigned, granted, conveyed, or licensed, and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- [X] No such person, concern, or organization exists.
- [ ] Each such person, concern, or organization is listed below.

Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Howard L. Elford		
NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
Howard L. Elford		
Signature of inventor	Signature of inventor	Signature of inventor
July 28, 1998		
Date	Date	Date

This is in accord with the submission of US Patent Application entitled THERAPEUTIC PROCESS FOR INHIBITING NF-κB, Cross Reference Provisional Application No. 60/054,230, filed 7/30/97, docket number HEBVR-5.

Certification under 37 CFR 1.10 (if applicable)

EH793205520US
"Express Mail" mailing number

July 28, 1998
Date of Deposit

I hereby certify that this application is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C., 20231.

Howard L. Elford
(Typed or printed name of person mailing application)

Howard L. Elford
(Signature of person mailing application)

PRINT OF DRAWINGS
AS ORIGINALLY FILED

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division

Application deficiencies found during scanning:

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